Mucosal Immunology 19 (2026) 1613–1623

Contents lists available at ScienceDirect

Mucosal Immunology

journal homepage: www.elsevier.com/mi

Iron-Dependent allergenicity of Alt a 1: A link between fungal nutritional immunity and allergic sensitization

Aila Fakhimahmadi [a,b,c], Karin Hufnagl [b,c,d], Ilir Hasanaj [a,b], Nathalie Szepannek [a,b], Gerlinde Hofstetter [a,b], Markus Wiederstein [e], Sebastian A Jensen [c,d], Markus Berger [a,c], Markus Gorfer [f], Clara E. Pogner [f], Rodolfo Bianchini [a,b,c], Isabella Pali-Schöll [a,b,c], Erika Jensen-Jarolim [b,c,d], Franziska Roth-Walter [a,b,*]

[a] Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Austria
[b] Messerli Research Institute, Department of interdisciplinary life sciences, University of Veterinary Medicine Vienna, Austria
[c] Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria
[d] AllergyCare Allergy Diagnosis, Private Clinic Döbling, Vienna, Austria
[e] Department of Biosciences & Medical Biology, University of Salzburg, Salzburg, Austria
[f] Bioresources Unit, Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, Tulln, Austria

A B S T R A C T

The major fungal allergen Alt a 1 from Alternaria alternata is linked to allergic asthma. We assessed its biological role in nutritional immunity to iron and its allergenic potential using *in silico*, *in vitro*, and *in vivo* approaches. Alternaria was cultured with or without iron and with quercetin to promote iron-quercetin (FeQ2) complexes, followed by Alt a 1 expression analysis, which revealed enhanced expression under iron deficiency and reduced levels by addition of quercetin. In silico and RBLsx38 mast cell degranulation assays showed that FeQ2 binding to Alt a 1 (holoAlt a 1) masked the IgE epitope Y87-D96 by promoting tetramer-formation of Alt a 1, reducing IgE binding and antigen-specific degranulation compared to ligand-free Alt a 1 (apoAlt a 1). In BALB/c mice, intranasal exposure to apo- or holoAlt a 1 before intraperitoneal Alt a 1/alum sensitization demonstrated that holoAlt a 1 exposure led to lower allergen-specific antibody titers, fewer mature antigen-presenting cells, increased regulatory T cells, and reduced allergic symptoms upon challenge compared to mice exposed to apoAlt a 1. Additionally, human PBMCs incubated with holoAlt a 1 exhibited fewer Th cells and plasmablasts, but more immature B cells with a higher iron content than those exposed to apoAlt a 1. These findings demonstrate that iron-poor conditions trigger nutritional immunity in Alternaria, increasing Alt a 1 expression, and that apoAlt a 1, through iron scavenging, has greater sensitizing capacity than the holo form, influencing immune responses relevant to allergic asthma.

One Sentence Summary: Iron-poor conditions activate nutritional immunity in *Alternaria*, increasing the expression of allergenic dimeric Alt a 1, whereas its iron-bound tetrameric form does not trigger an immune response and thus prevents allergy development.

Introduction

About a quarter of people in the Western world suffer from allergies, making it essential to understand why only certain allergen families emerge as major allergens and why only some individuals become sensitized to them.

Alternaria alternata is a fungus usually residing in plants, where it can live in an asymptomatic symbiotic relationship and contribute to plants' health and stress tolerance[1]. However, under certain circumstances Alternaria can turn into an opportunistic plant pathogen, invading the host via necrotic tissue[2] and deriving its nutrients from dead organic matter. This shift from symbiont to pathogen appears to occur by plant stress, in which one known stress factor is nutrient deficiency[3].

Likewise in humans, Alternaria may also settle in the skin[4,5] and the conjunctiva in an asymptomatic manner[6] and usually only cause disease in immunocompromised individuals[5,7,8].

Alternaria alternata is also strongly related to severe allergic asthma, with 80 % of affected individuals being sensitized solely to its major allergen Alt a 1[9–11], which suggests a driving role of this protein in the shift from the apathogenic to the pathogenic state.

In general, fungal colonization and microbial overgrowth in the human host is prevented by inducing unfavorable living environment via innate mechanisms[12]of which nutritional immunity is one of the most evolutionary conserved ones. Here the induction of nutritional immunity for specific nutrients such as iron[13–15] leads to cellular iron withholding and accumulation in ferritin and blocking nutrient

* Corresponding author.
E-mail address: franziska.roth-walter@vetmeduni.ac.at (F. Roth-Walter).

https://doi.org/10.1016/j.mucimm.2025.11.006
Received 10 July 2025; Accepted 11 November 2025
Available online 13 November 2025

absorption and systemic circulation to limit nutrients for pathogen growth. Pathogens counteract host-derived stress or interfere with humans' iron homeostasis to ensure virulence during infection[16].

As iron is essential for Alternaria, this fungus has evolved several sequestration strategies to capture the precious nutrient including the production of siderophores[17–22].

In previous studies, we demonstrated that the major allergen Alt a 1 has outstanding affinities to chelated iron (similarly high as high-affinity antibodies for their antigen). Binding to chelated iron by Alt a 1 promoted an apathogenic tetrameric/hexameric conformation[23,24] which suggests that indeed Alt a 1 may participate in iron nutritional immunity[25] and that this feature is mechanistically important for allergic sensitization.

Here, we went a step further and assessed the natural function of Alt a 1 by growing Alternaria in iron-poor conditions and conducting in vivo experiments. Under iron-poor conditions, we identified increased Alt a 1 expression in the fungi, while one major IgE epitope of Alt a 1 became exposed. Indeed, the results emphasized that patients' immune system generated IgE particularly under iron-deficient conditions. Additionally, only the iron-deprived version of the fungal allergen had greater sensitization capabilities *in vivo* and primed the immune system *in vitro* and *in vivo*.

Results

Enhanced Alt a 1 expression in Alternaria grown under iron deficient conditions

Nearly every organism is dependent on iron[26,27]. We hypothesized that one of Alternaria's iron-sequestration strategies requires the major allergen Alt a 1. Alternaria was thus grown under different conditions: either in medium with iron or depleted of iron by BPS (bathophenanthroline disulfonate) or in iron-rich medium with added quercetin to achieve iron −quercetin (FeQ2) complexes and help the fungi in iron sequestration[21,22,28,29]. As depicted in Fig. 1 A, deprivation of iron impacted Alternaria growth, while the addition of quercetin improved the bioavailability of iron and led to melanisation of the mycelium. Iron-deficiency had a negative impact on the biomass (Fig. 1B) and no spores were produced under the applied conditions. Interestingly, Alt a 1 has been described to be exclusively found in the melanin of the spore walls[30]. Analysis of the Alternaria extract in Fig. 1C revealed an enhanced recognition of dimeric Alt a 1 under iron-deprived conditions in the fungal pellets, while addition of quercetin significantly inhibited its expression. In the supernatant faint bands of dimeric and tetrameric Alt a 1 was observed, when Alternaria was cultured only in iron-poor conditions. A similar IgE-binding profile to the Alternaria extracts was also measured in ELISAs with sera from Alternaria allergic volunteers showing reduced IgE binding in iron-sated conditions.

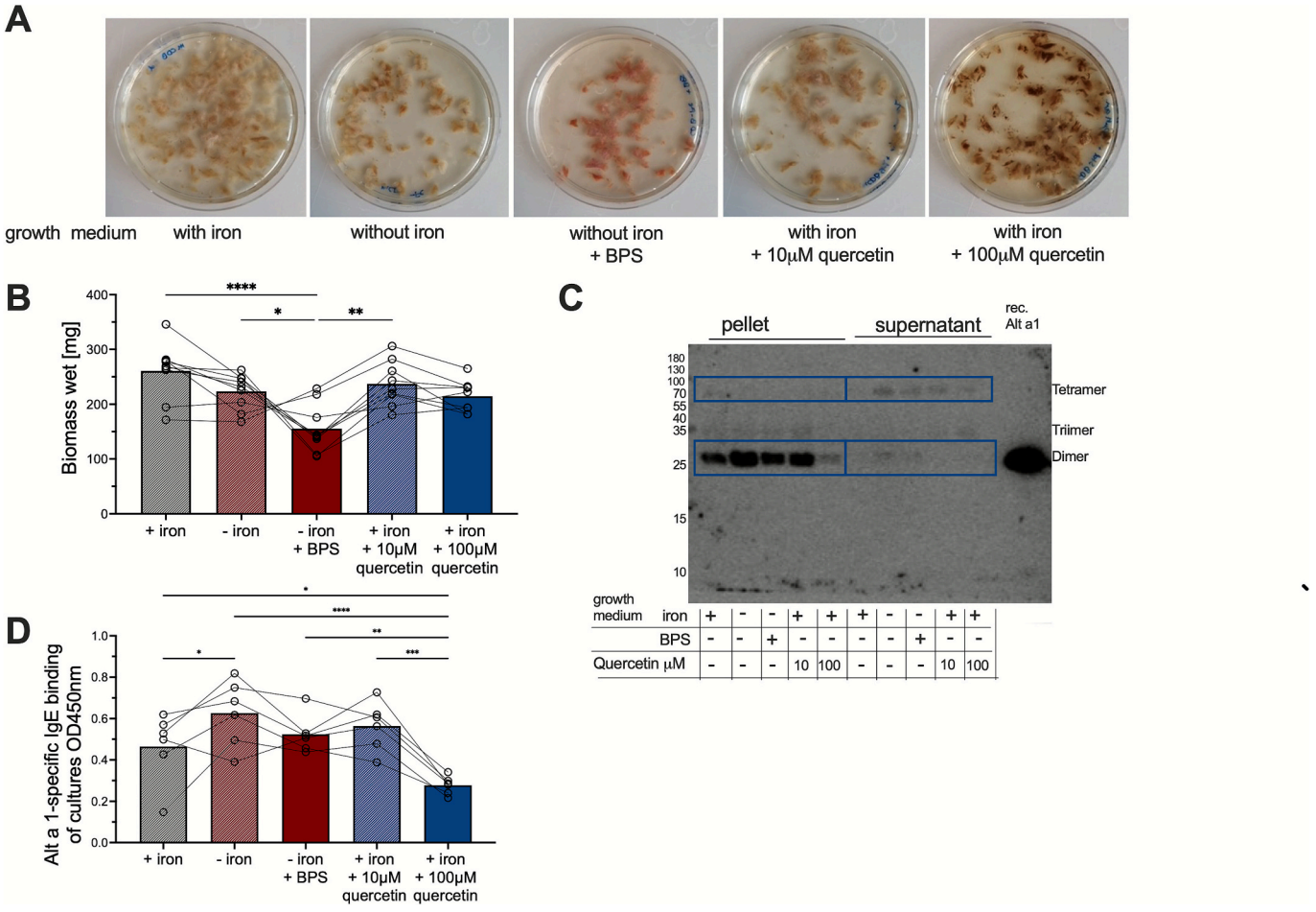

Fig. 1. Enhanced IgE-recognition of Alt a 1 in Alternaria grown under iron deficient conditions. (A) Representative images of fungal colonies; (B) Biomass, (C) Alternaria-specific-IgE binding of sera in Western Blot to pellet and supernatants and (D) ELISA of Alternaria alternata cultures grown on broth containing iron (+) or without (−) iron, without iron and addition of the iron chelator bathophenanthrolindisulfonic acid (BPS) or in medium with iron and containing 10 or 100 µM quercetin (+iron + quercetin), respectively. Summary of three independent experiments are shown; groups were compared by RM one-way ANOVA followed by Holm-Sidak's multiple comparison test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

To sum up, iron-deficiency led to significantly enhanced Alt a 1 expression in the dimeric form, despite the loss in biomass in Alternaria. In contrast, addition of quercetin fostered a tetrameric state that was less recognized by patients' IgE antibodies. The data clearly link iron-deficiency with Alt a 1 and a function in nutritional immunity.

Alt a 1's binding to chelated iron masks a major IgE B cell epitope affecting IgE binding and mast cell degranulation

The natural ligand of Alt a 1 has been isolated and shown to possess a quercetin-core structure. Quercetin itself, is known for its strong ability to chelate iron[29] and is attributed anti-inflammatory properties[31]. To discern the loading state, the empty protein without ligand is named apoAlt a 1, whereas the counterpart binding to ligand is named holoAlt a 1. Based on previous research[31–34], we hypothesized that Alt a 1 binding to iron chelated via quercetin would be perceived less allergenic. Indeed, in silico analysis revealed that binding to the iron-complexes particularly masked one of the four described IgE-epitopes[35,36], namely IgE epitope Y87-D96 (blue) that fosters tetramer formation (Fig. 2A-C). Consequently, IgE-binding was significantly inhibited (up to 79 %) in ELISAs (Fig. 2D) and in blocking experiments (60 % inhibition with apo-, but not holoAlt a 1, Fig. 2E). The reduced IgE-binding achieved with holoAlt a 1 emphasizes that patients' IgE antibodies were generated at a time-point where this major IgE epitope was not masked and Alt a 1 was prevalent in its apo- and dimeric form (Fig. 2 D-E). Moreover, in mast cell experiments using RBLsx cells, (rat basophilic leukemia cells stably transfected with human high-affinity IgE receptor) that were sensitized with sera from *Alternaria* allergics, IgE cross-linking with holoAlta 1 significantly impaired antigen-specific degranulation (−47 %) when compared to apoAlt a 1 (Fig. 2F). Thus, our data show that the accessibility of IgE binding regions varies with oligomerization, with Y87-D96 masked in the tetrameric form, resulting in reduced IgE binding and impaired antigen-specific mast cell degranulation compared to apoAlt a 1.

Less symptoms in holoAlt a 1 –preexposed mice in vivo

To discern *in vivo* relevance how dimeric Alt a 1 (apoAlt a 1, without iron-quercetin) or tetrameric holoAlt a 1 (with iron-quercetin complexes) affect sensitization, mice were nasally pretreated with apo/holoAlt a 1 or the controls alone, before all mice were sensitized with the allergen and alum intraperitoneally. Subsequently, symptoms were

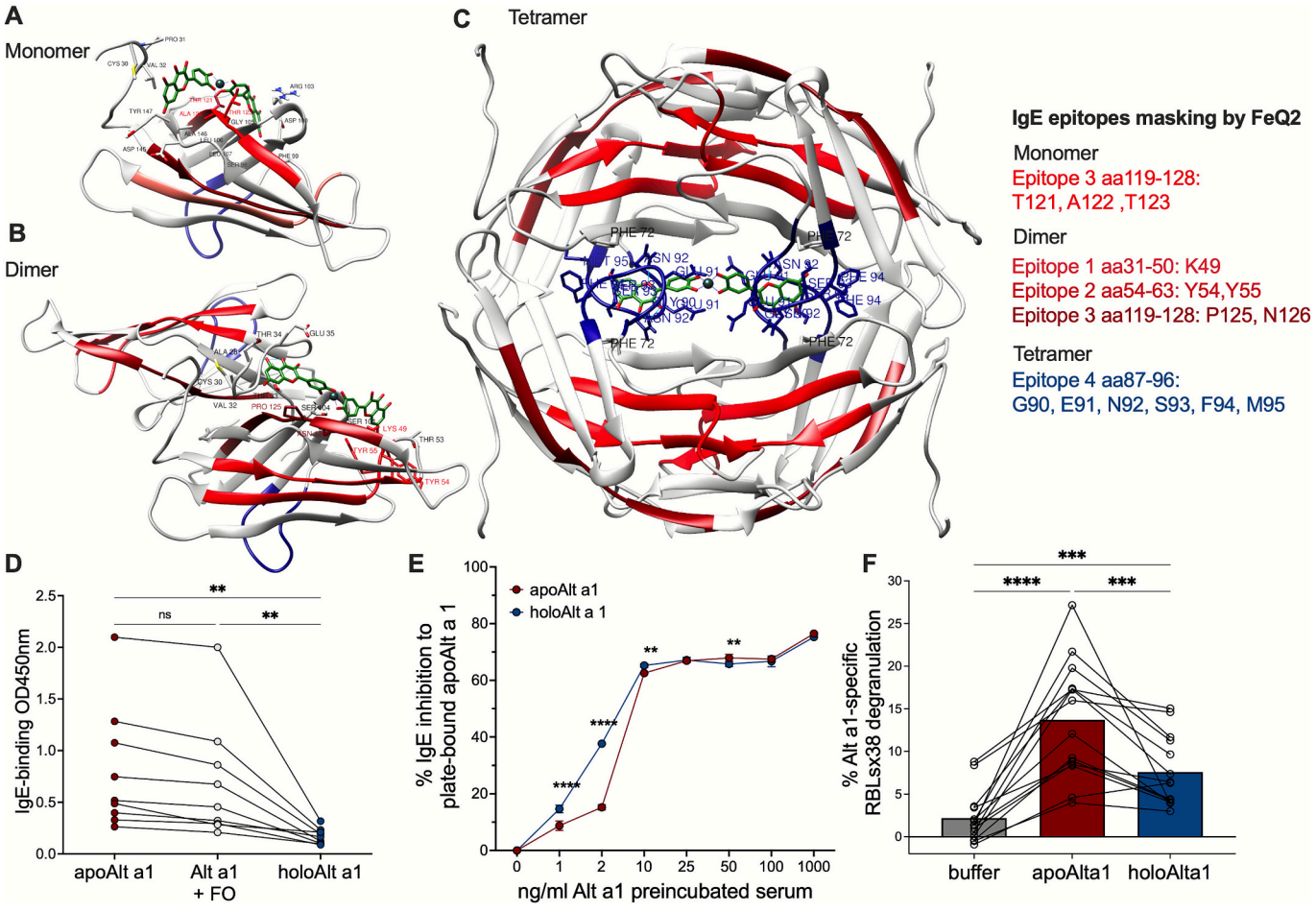

Fig. 2. Binding of Alt a 1 to iron-quercetin complexes masks IgE epitopes affecting IgE binding and mast cell degranulation. (A) Mono-, (B) di- and (C) tetrameric structure of Alt a 1 incorporating iron(quercetin)2 FeQ2 (sticks with carbons in green, oxygens in red, and iron shown as a grey sphere) as cartoon and with major IgE B cell epitope (K41-P50), epitope (Y54-K63) and epitope (V119-D128) marked in light and dark red hues, respectively and epitope (Y87-D96) marked in blue. Residues within a 4Å distance from any atom of FeQ2 are shown as sticks. (D) Alt a1-specific IgE binding of individual subjects allergic to Alternaria alternata (n = 10) to Alt a 1 without ligand (apoAlt a 1) or in conjunction with ferroxamine FO (control iron-chelate, to which it does not bind to) or with FeQ2 (holoAlt a 1). (E) Blocking experiments with pooled Alternaria allergic sera incubated with increasing doses of apo- (red line) or holo Alt a 1 (blue line) and binding to plate-bound apoAlt a 1. (F) Degranulation of RBLsx38 cells sensitized with pooled sera of allergic subjects to Alternaria alternata and crosslinked with 100 ng/ml apo- or holoAlt a1. Statistical analysis was performed with RM one-way ANOVA followed by Holm-Sidak's multiple comparison test for (D) and (F) and RM-two-way ANOVA with Dunnett's multiple comparison test for (E). **p < 0.01, ***p < 0.001, **** p < 0.0001.

evoked by challenging mice with the allergen alone intraperitoneally and monitoring body temperature drop (as a sign for anaphylaxis) and scoring the symptoms (Fig. 3A). Indeed, mice nasally preexposed to holoAlt a 1, but not apoAlt a 1, had significantly less anaphylactic symptoms as measured by a higher body core temperature and lower symptom scores than mice previously exposed to apoAlt a 1 or controls (Fig. 3B and C).

Less antibodies and cytokines in mice preexposed to holoAlt a 1 in vivo

When we assessed the Alt a 1 −specific humoral immune response, mice sensitized to holoAlt a 1 had significantly less specific Alt a 1 antibodies (IgE, IgG1 and IgG2a) than those sensitized to apoAlta 1. This was true both prior (Fig. 4A) as well as post ip-sensitation with Alt a1 (Fig. 4B), which emphasizes the overall lower sensitizing abilities of holoAlt a1 compared to apoAlt a1.

Overnight cultures of unstimulated splenocytes showed an overall higher activation status in mice exposed to apoAlt a1, as indicated by increased IFNγ. Notably, both apoAlt a1 and holoAlt a1 exposed groups also exhibited elevated IL-10 levels, suggesting activation of regulatory pathways alongside the proinflammatory response (Fig. 4C). In short, less sensitization and a lower activation status was observed in animals pre-exposed to holo-, than to apoAlt a 1.

Reduced antigen presentation and more Tregs upon holoAlt a 1 exposure in vivo

The next step was to assess phenotypical characteristics of immune cells in the different treated groups.

The first cells processing proteins and crucial in immune activation are antigen-presenting cells (APCs)[37] with antigen presentation in the absence of co-stimulatory molecules known to result in anergy, a state of immune unresponsiveness[31].

In line with the humoral data and the lower symptom levels in mice exposed to holoAlt a 1, also the relative numbers of mature CD11 + CD86 + MHCclassII + dendritic cells and the relative levels of costimulatory molecules CD86 + MHCclassII + cells were significantly reduced (Fig. 5A). In contrast, relatively more regulatory CD4 + CD25 + Foxp3 + T regulatory cells, that are important in suppressing effector T cells and maintaining tolerance[38], were detected than in mice exposed to apoAlt a 1.

Thus, holoAlt a 1 was less immunogenic and promoted immune resilience.

Less Th-cells and mature B cells in PBMCs incubated with holoAlt a 1

In a next step, we assessed in greater detail impact of apo- and holoAlta1 in human peripheral blood mononuclear cells (PBMCs) obtained from individuals with *Alternaria* allergy. We focused on the B- and T cells compartments as lower antigen-presentation upon holoAlt a 1 incubation in the monocytes has already been described[23]. Thus, we stimulated PBMCs overnight either with empty apoAlt a 1 or binding to iron-quercetin chelates (holoAlt a 1).

Incubation with holoAlt a1 − though having no impact on the overall relative numbers of T cells (Fig. 6A)- significantly lowered the relative numbers of Th-cells (Fig. 6B). However, no relative changes occurred in the Th2 (Fig. 6C), or Treg (Fig. 6D) compartment. In line, with the lower Alt a 1 specific antibody levels observed in the murine models, relatively less CD3-CD19-CD27 + CD38 + plasmablasts, but not CD19 + CD27 + CD38- memory B cells were detected[39], while particularly immature B cells were fostered. Interestingly, B cells incubated with holoAlt a 1 depicted a lower sensitivity to stimuli as measured by lower CD19 expression, that went along with an increased labile iron content.

To sum up, the results showed that Alt a 1, when combined with the

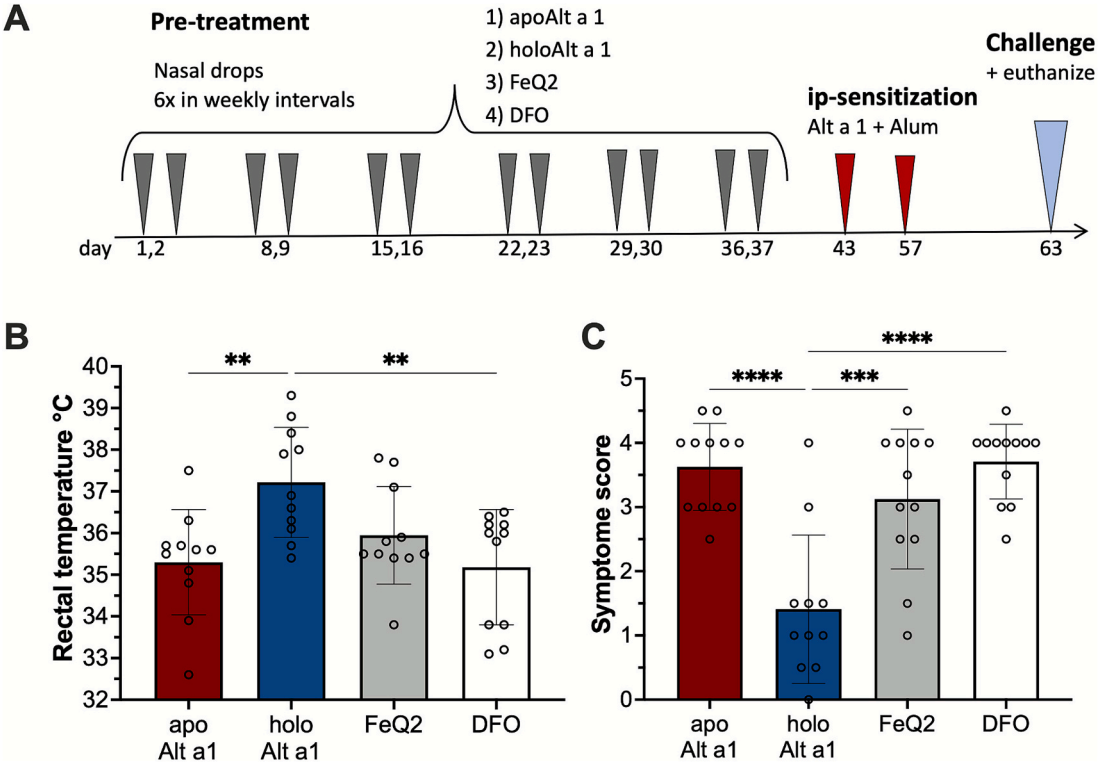

Fig. 3. Reduced clinical reactivity in mice sensitized to Alt a 1, when preexposed to holo- but not apoAlt a 1. (A) Treatment scheme: Mice received intranasal drops (6 times in weekly intervals on two consecutive days) of 1) apoAlt a 1 (Alt a 1 + deferoxamine DFO) 2) holoAlt a 1 (Alt a 1 + FeQ2), or sham-treatment with 3) FeQ2 or 4) DFO) alone, before intraperitoneal (ip) sensitization with Alt a 1 (twice in conjunction with alum) and subsequent ip-challenge with Alt a 1. Summary of individual response of two experiments (B) Rectal temperature and C) symptom score. Data were compared with ordinary one-way ANOVA and Tukey's multiple comparison test. **p < 0.01; **p < 0.001; ****p < 0.0001;

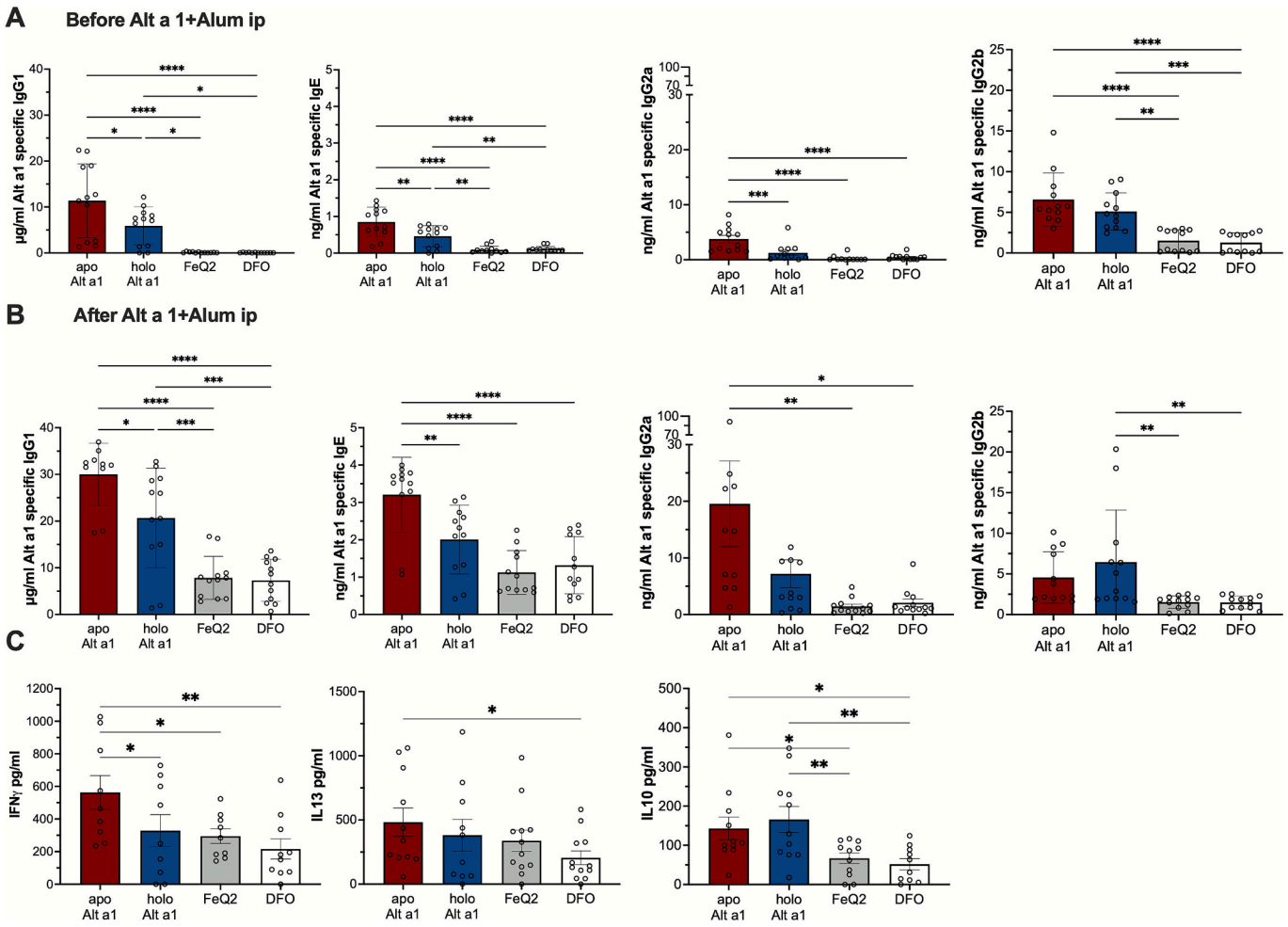

Fig. 4. Reduced sensitization upon holoAlt a 1 exposure (A) Alt a 1-specific IgG1, IgE, IgG2a and IgG2b antibody-profile in mice treated 6x intranasally with apoAlt a 1 (Alt a 1 + deferoxamine DFO); holoAlt a 1 (Alt a 1 + FeQ2) or with the controls FeQ2 or DFO) alone, (B) and after sensitizing twice ip with Alt a 1 and alum (C) IFNg, IL13 and IL10 release of splenocytes from the different treated groups incubated overnight in iron-free media. Summary of individual responses of two independent performed experiments. Data were compared by ordinary one-way ANOVA following Tukey's multiple comparison test for the antibodies and following uncorrected Fisher's LSD with single pooled variance for the cytokine-responses. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

micronutrient iron-quercetin, promotes rather a regulatory immune phenotype and is not allergenic, as it is less able to generate IgE antibodies and in the sequelae allergic symptoms.

Discussion

Nutritional deficits are not trivial and are also relevant in westernized countries. Iron-deficiency is associated with all-cause morbidity and mortality[40–42] in humans and evokes nutritional immunity in nearly all organisms[13,27,43–46]. Upon nutritional immunity, humans predominantly hide the vital mineral in the liver and macrophages, blocking redistribution of iron and dietary iron absorption, which result in lower systemically iron-levels[13,47]. Iron-deficiency alone is sufficient to generate a Th2 environment in individuals[44], thus is able to set the stage for allergic sensitization, and is also causal for a lower lung function[48,49] in asthmatics.

Alternaria strongly relates to allergic asthma with usually allergic sensitization to Alternaria[9–11] occurring only against one single protein, namely the major allergen, Alt a 1[50]. The fact that Alt a 1 also has an outstanding binding affinity to chelated iron and also demonstrates some proteolytic activities in a nutrient-deprived environment[51] suggested to us that indeed Alt a 1′s iron-binding may contribute to its pathogenicity and allergenicity. Notably, a recent plant study demonstrated that Alt a 1 acts as the pathogenic effector driving both fungal persistence and the hypersensitive response associated with apple leaf spot in apple[52].

Indeed, also for Alternaria – a fungus, commonly found in soil and plants[53] – iron is a key nutrient for the fungus's growth, development and virulence[27,47]. In our studies, we chose as chelator for iron, quercetin, as it is a flavonoid present in plants. Additionally, the natural isolated ligand for Alt a 1 has been described as a methylated quercetin-like flavonoid, notable for its dual function of inhibiting the growth of plant roots and neutralizing reactive oxygen species[54].

Upon iron-limitation other opportunistic pathogenic fungi, such as *Aspergillus fumigatus*[47] or *Candida albicans*[55], are known to become more aggressive and express virulence factors such as adhesins, proteases and stress response proteins, and secrete siderophores. Candida also form hyphae which contribute to its ability to colonize host tissues and evade immune responses[55].

Here, we provide for the first time strong evidence that Alt a 1 participates in nutritional immunity[15,24]. Already, iron-poor-conditions increased Alt a 1 expression in Alternaria culture and resulted primarily in the generation of a dimeric, nutrient-deprived version of Alt a 1 (apoAlt a 1). Dimeric apoAlt a 1 was allergenic and was recognized by patients' IgE antibodies, whereas recognition of the tetrameric nutrient-binding version was significantly lower. Among the four known IgE-epitopes, iron-quercetin complex FeQ2 fully masked IgE epitope Y87-D96, thereby blocking IgE binding nearly completely. These findings

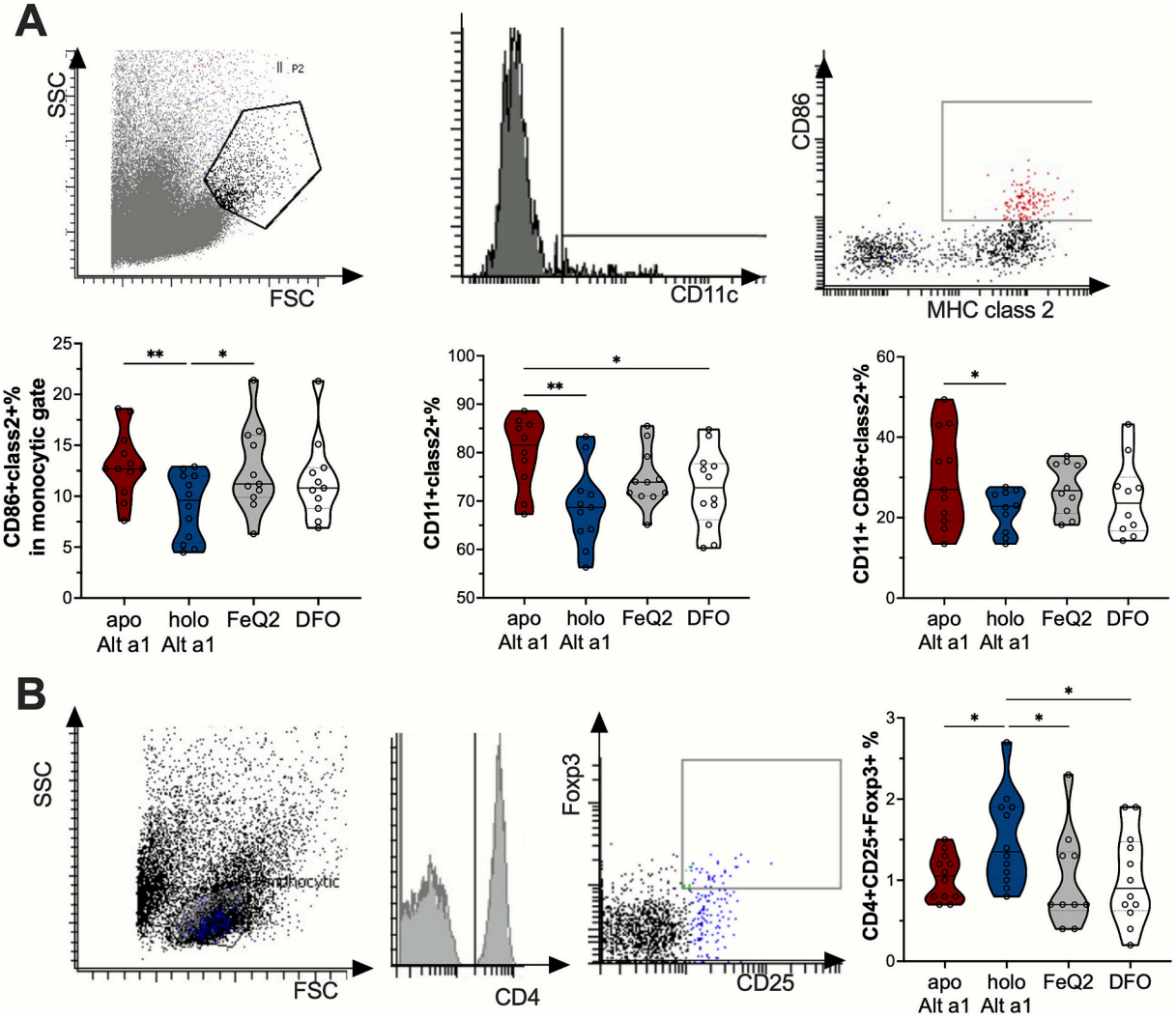

Fig. 5. Reduced antigen presentation and more Tregs upon holoAlt a 1 exposure. Splenocytes of mice intranasally exposed to apoAlt a 1 (Alt a 1 + deferoxamine DFO); holoAlt a 1 (Alt a 1 + FeQ2) or to the controls FeQ2 or DFO alone and subsequently sensitized twice with Alt a 1 in conjunction with alum were flow-cytometrically assessed. (A) Gating strategy for dendritic cells in splenocytes and relative numbers of CD86 + MHCclassII + cells, relative CD11c + MHCclassII positive and CD11 + c CD86 + MHCclassII + cells (B) Lymphocyte gating of CD4 + cells for Foxp3 + CD25 + and relative numbers thereof. Data were compared by ordinary one-way ANOVA following uncorrected Fisher's LSD with single pooled variance *p < 0.05; **p < 0.01.

are very relevant as they imply that only under nutrient-, iron-depleted conditions allergic sensitization occurred as otherwise no IgE antibodies against the major B cell epitope Y87-D96 could have been generated.

A very similar pattern to the *in vitro* data was observed in vivo, in which nasal application of holoAlt a 1 compared to dimeric apoAlt a 1 resulted in significantly lower levels of antigen-specific antibodies, a general lower immune activation status, with less mature antigen-presenting cells and more regulatory cells, resulting in an overall reduced symptom burden of allergen-challenged mice.

The observed reduction in antibody titers and increase in Tregs could, in principle, reflect either a general immunosuppressive effect of the iron–quercetin or an antigen-specific tolerogenic mechanism. Our data, however, indicate that the effect is primarily antigen-specific. FeQ2 alone is not efficiently taken up by cells, whereas Alt a 1 acts as a carrier that enables FeQ_2 uptake. Once internalized, the Alt a 1–FeQ_2 complex alters Alt a 1 processing and presentation, thereby reducing antigen-specific immune activation. In parallel, FeQ_2 transiently increases the labile iron pool, and quercetin activates the aryl hydrocarbon receptor (AhR), both of which are known to promote regulatory and anti-inflammatory pathways[24]. Together, these mechanisms favor antigen-specific tolerance rather than a general suppression of immune responses. The outcome likely depends on antigen type and exposure context: for food proteins encountered in large amounts, iron–polyphenol interactions may induce a broader hyporesponsiveness, whereas for environmental allergens like Alt a 1, which act locally and intermittently, a localized, antigen-dependent tolerance is more plausible.

Mechanistically, the reduced synthesis of antibodies could be replicated *in vitro* using primary human immune cells. Also in this setting, incubation with holoAlta1 alone was sufficient to reduce the numbers of activated B cells and plasmablasts, while more immature B cells with lower sensitivity were fostered. These immature B cells had also a significantly larger labile iron pool[56], all indicators for the lower activation status. In parallel, holoAlt a 1 exposure led to a lower frequency of Th2 cells, whereas the overall proportion of $CD3^+CD4^+$ T cells remained unchanged, indicating modulation of T-cell activation rather than depletion of this population. Together, these findings support an early tolerizing immune response consistent with iron- and AhR-dependent regulatory programming.

Literature search and our previous studies revealed that also other allergens share iron-binding properties that decrease their allergenicity as reported for peanuts (Ara h 1 – 7S, Ara h 3 –11S protein)[57,58], egg

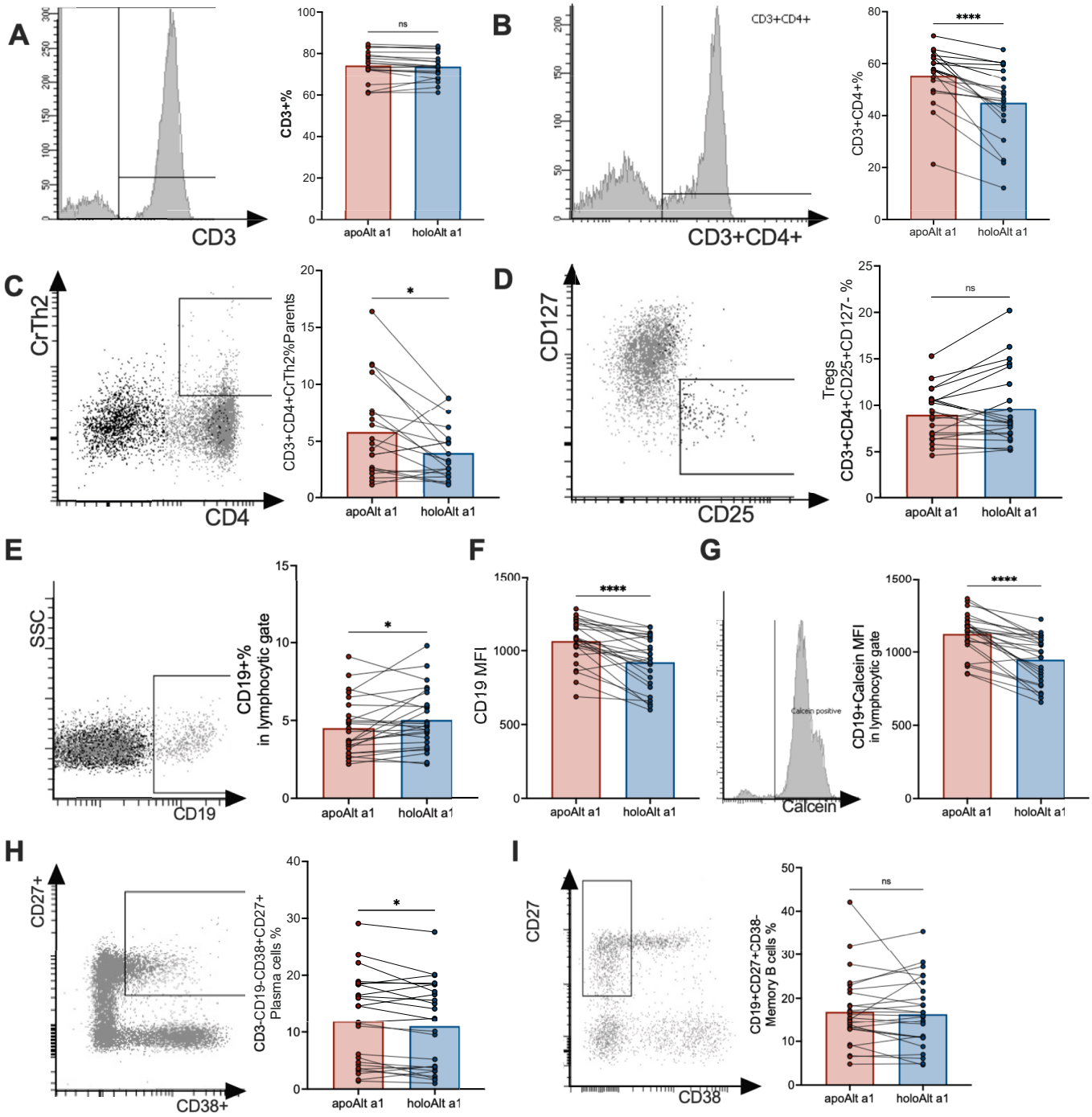

Fig. 6. PBMCs incubated with holoAlt a 1 have less mature B cells and less Thelper cells. PBMCs isolated by density gradient were incubated overnight in iron-free media and in the presence or absence of apoAlta1 (red bars) or holoAlta1 (blue bars) before analysis of intracellular iron by quenching of the calcein signal and phenotypic changes in T (n = 22) and B cells (n = 26). Summary of relative numbers of (A) CD3+, (B) CD3 + CD4 + cells, (C) Th2 (CD4 + CrTh2 + cells) and (D) regulatory T cells (CD3 + CD25 + CD127-). For B cells: relative numbers of (E) CD19 cells and mean fluorescence intensity of the (F) CD19 as well as the (G) Calcein signal of CD19 positive cells. Relative numbers of (H) CD3-CD19-CD27 + CD38 + plasmablast and (I) CD19 + CD27 + CD38- memory B cells. Data from 26 subjects are shown. Groups were compared by paired T test, except for Tregs, Th2 and plasma cells, in which Wilcoxon matched-pairs signed rank test was employed due to non-parametric distribution of the data. *p < 0.05; **p < 0.01, ****p < 0.0001. n.s. not significant.

(Gal d 3)[59], milk (Bos d 5)[31,33] and birch pollen (Bet v 1)[32,34]. This reduced allergenicity does not seems only to be restricted to iron as also binding to vitamins such as vitamin A[15,60,61] renders proteins less allergenic.

As such, the allergenicity of potential allergenic proteins appears to be a context-dependent phenomenon as also demonstrated here with Alt a 1 *in vitro* and *in vivo*. Their function can change depending on nutrient availability, from useful nutrient carriers in a nutrient-rich environment to potential allergens and nutrient-scavengers under nutrient-poor conditions.

These results imply that the atopic state in a person is dependent on the nutritional status as nutrient deficiencies do prime the immune system[27,43,44], facilitate IgE antibody formation (to proteins capable of scavenging nutrients and contribute to disease severity in atopic

sufferers[45,46,62–67]. Therapeutical strategies for allergics thus should consider on the one hand the administration of allergens with nutritional ligands as chelated iron in allergen immunotherapy[68] and on the other hand the assessment and correction of micronutritional deficiencies in the allergic individual[44] itself.

Materials and methods

Ethical approval

Volunteers (n = 27) donated blood after providing written informed consent. Experiments with blood samples and PBMCs from *Alternaria*-allergic patients were approved by the ethics committee of the Medical University of Vienna and conducted in accordance with the principles of the Helsinki Declaration of 1975 (EK number 1318/2021is). All subjects gave their full written informed consent. Volunteers were considered allergic, when specific IgE against Alt a 1 was detected via ALEX®-test (Macro Array Diagnostics, Vienna, Austria).

Animals

Female, inbred 7-week old BALB/c mice, were obtained from Charles River Laboratories (Bad Königshofen, Germany), kept under conventional housing, and treated according to European Union rules of animal care with the permission of the Austrian Ministry of Sciences (BWF 2021–0.550.211). The combined results from two independent experiments with each n = 6/group are shown.

Generation of the Pichia pastoris strain expressing the Alternaria main allergen Alt a 1

E. coli DH5α strain was grown in low-salt Luria Bertani broth and transfected with the pPICZα A vector (Invitrogen, Waltham, MA, United States) with the gene of interest (General Biosystems, Morrisville, NC, USA) then selected with Zeocin™ antibiotic (Invitrogen, United States). The plasmid DNA was isolated and checked with PCR using AOX1 gene forward and reverse primers: 5′ AOX1 5′-GACTGGTTCCAATTGACAAGC-3′ and 3′ AOX1 5′-GCAAATGGCATTCTGACATCC-3′. Next, the plasmid DNA was transferred to the Pichia pastoris X33 strain using electroporation and cultured on Yeast Extract Peptone Dextrose Medium (YPD) and + Zeocin™ plates. To confirm the integration of the expression construct into the genome of the Pichia transformants, a colony PCR using plasmid and gene-specific primers was performed. For further confirmation, the DNA was sent for DNA sequencing.

Positive colonies were selected and inoculated on BMGY (1 % yeast extract, 2 % peptone, 100 mM potassium phosphate, pH 6.0, 1.34 % YNB, 4 × 10 − 5 % biotin, and 2 % glycerol) and Zeocin™ (25 μg/ml). They were cultured at 27–28 °C in a shaking incubator (250–300 rpm) until the culture reached an OD600 of 2–6. The cells were then harvested, and to induce expression, the cell pellet was resuspended in BMMY medium (the same as BMGY with 1 % methanol instead of glycerol) using 1/5 to 1/10 of the original culture volume. 100 % methanol was added to a final concentration of 1.5 % every 24 h to maintain induction.

The final harvesting was done after 144 h. The supernatant was collected and purified with anion exchange chromatography and 10 mM Na-phosphate pH 7.5. Concentration was measured by the Bicinchoninic acid assay (BCA), and the EndoZyme recombinant Factor C Endotoxin Detection Assay was used for endotoxin measurement.

Recombinant Alt a 1 used in the experiments contained 1.9 endotoxin units per mg protein.

Generation of apo and holoAlt a 1

Recombinant Alt a 1 was dialyzed 3 times against 10 μM deferoxamine mesylate salt. Further dialysis against deionized water was performed to generate apoAlt a 1. Holo Alt a 1 was generated by forming iron–quercetin (FeQ2) complexes with quercetin (Sigma 1592409) and iron (iron standard AAS, Sigma) at a ratio of 2:1, adjusting the pH to 7.2 with 10 mM NaOH and adding apoAlta 1 to a final concentration of 1 μM Alt a 1, 2 μM quercetin, and 1 μM iron.

Alternaria growth and extraction

To produce Alternaria alternata (SC08) extracts, 20 mL liquid mCDB media (4 % glucose, 0.1 % yeast extract, 0.1 % $NaNO_3$, 0,.025 %, NH_4Cl, 0.1 % KH_2PO_4, 0.025 % KCl, 0.025 % NaCl, 0.05 % $MgSO_4$ ($7xH_2O$), 0.001 % $FeSO_4$, 0.001 % $ZnSO_4$, 1.5 % agar) in 90 mm petridishes was inoculated with 10 μL spore suspension and grown for 3 days at 28 °C.

To harvest the fungus, the culture was filtrated over 0.45 μm nitrocellulose filter (Pall) and washed two times with 50 mL sterile iron exchanged water. The pellet was transferred to a new 90 mm petri dish, with 20 mL media, with different additives, and grown another day at 28 °C. The mycelium was cut into smaller pieces using a spatula to destroy the pellet. For each condition 6 plates were prepared, 3 for protein extraction and 3 for RNA analysis. This culture was also harvested by filtration and the mycelium and supernatant analyzed.

Different media were used in the growth studies: mCDB; mCDB without 0.001 % $FeSO_4$; mCDB without 0.001 % $FeSO_4$, with 250 μM BPS (Sigma B1375 Bathophenanthrolinedisulfonic acid disodium salt hydrate); mCDB with 10 μM quercetin (Sigma Q4951); mCDB with 100 μM quercetin

Protein harvest

For protein analysis, supernatants were centrifuged to remove remaining mycelium. The clear supernatants were frozen with liquid nitrogen and lyophilized for two days. The mycelium in the plate was harvested by vacuum filtration and the biomass was weighed and transferred to tubes containing 17 magnetic beads and 500 μL 1:10 diluted protease inhibitor buffer (1:10 dilution of EDTA free Protease Inhibitor, Sigma Aldrich).

For protein extraction the mycelia samples were beat beaded (FastPrep, MP Biomedicals) 3 times for 20 s at 4.5 m/s with a brake of 40 s between the different runs and incubated at 4 °C and 16 rpm overnight. They were centrifuged at 14000 rpm and 4 °C for 10 min and the supernatant was transferred to 1.5 mL centrifuge tubes, and 1:10 and 1:100 dilutions in BioScience water were prepared for further protein measurements.

The lyophilized samples from supernatant were resuspended in 1 mL 12 % TCA (Trichloroacetic acid) in BioScience water. Samples were centrifuged at 16000 rpm and pellets were dried on a thermoblock at 55 °C for 30 min and dissolved in 125 μL 1:10 protease inhibitor buffer (1:10 dilution of EDTA free Protease Inhibitor, Sigma Aldrich). Samples were put to a thermoblock at 95 °C and shaken for 10 min and afterwards centrifuged at 16000 rpm and 4 °C for 15 min. The supernatants were transferred to new 1.5 mL centrifuge tubes and 1:10 and 1:100 dilutions in BioScience water were prepared for further protein measurements.

SDS page and immunoblotting

Apo Alt a 1 and pellets as well as supernatants of Alternaia extracts were mixed with 4x non-reducing sample buffer and 12 μg/slot was applied on two SDS–PAGE 4–20 % gradient gels (Biorad Tokyo, Japan), run at 90 V for about 1 h. Subsequently gels were blocked and incubated with serum pool (1:10 diluted) of Alternaria allergic patients and followed by incubation with secondary antibody HRP-labelled anti-human IgE antibody (Invitrogen United States). Subsequently, ECL substrate (clarity TM Western ECL Substrate, BioRad, Tokyo, Japan) was added and the luminescent degradation product was detected with the ChemiDoc™Touch Imaging System (BioRad, Tokyo, Japan)[32].

Alt a 1 detection in Alternaria extracts with patient's sera

To detect Alt a 1, MaxiSorp 96-well plates were coated overnight at 4 °C with the Alternaria extracts of Alternaria grown under the different conditions. After blocking (1 % BSA in 0.89 % NaCl and 0.05 % Tween 20), wells were incubated with individual sera of Alternaria allergic patients (diluted 1:10 in 0.89 % NaCl/0.05 % Tween 20) at 4 °C. Bound IgE was detected using horseradish-peroxidase-conjugated goat anti-human IgE antibody (diluted 1:4000) and tetramethylbenzidine (TMB) as the substrate. Color development was stopped with sulfuric acid, and the optical density at 450 nm was measured using a microplate reader.

In silico analysis of Alt a 1

The atomic coordinates of Alt a 1 were obtained from the high-resolution (1.9 Å) crystal structure with Protein Data Bank (PDB) code 3V0R[69]. Water molecules, ligands, cofactors, and ions were removed from the structure. The receptor and ligand structures were prepared using the reduce v3.23[70] and ADFR software suite[71]. Protein-ligand docking calculations were carried out with the stand-alone version of AutoDock Vina v1.2.3-mod[72]. Figures and visualizations were created using UCSF Chimera[73].

Alt a 1 –specific IgE ELISA

Two μg/mL of apoAlt a 1, holoAlt a1-FeQ2, Alt a 1-Q2 or Alt a 1-FO (ferroxamine) diluted in 0.89 % NaCl were coated (100 μL per well) overnight at 4 °C, blocked for 2 h at room temperature with 1 % BSA in 0.89 % NaCl containing 0.05 % Tween 20 (200 μL per well), before incubated with 100 μL of diluted serum from birch-pollen allergic and non-allergic subjects (1:10 diluted in 0.89 % NaCl/0.05 % Tween-20) overnight at 4 °C. Bound IgE was detected with horseradish–peroxidase-conjugated goat anti-human IgE antibody (Invitrogen A18793, Waltham, MA, USA) diluted at 1:4000 in 0.89 % NaCl containing 0.05 % Tween-20 and using tetramethylbenzidine (eBioscience, San Diego, CA, USA) as a substrate. Color development was stopped with 1.8 M sulfuric acid. The optical density was measured at 450 nm by using an Infinite M200Pro microplate reader (Tecan, Grödig, Austria). Between the steps, rigorous washing was performed with 0.89 % NaCl containing 0.05 % Tween-20. Data are shown as a percentage normalized to apoAlt a 1 IgE binding levels or as OD at 450 nm.

Inhibition ELISA

ApoAlt a 1 (0.06 μM) were coated overnight at 4 °C, before blocking at room temperature for 2 h. Pooled serum from 15 Alternaria allergic subjects preincubated with increasing doses of apo- or holoAlt a 1 (1,2,10,25,50,100, 1000 ng/ml), was added overnight, before detecting bound IgE with horseradish–peroxidase-conjugated goat anti-human IgE antibody (Invitrogen A18793, diluted 1:4000 in 0.89 % NaCl containing 0.05 % Tween-20) and using tetramethylbenzidine (eBioscience) as substrate. After 20 min color development was stopped by adding 1.8 M sulfuric acid and optical density was measured at 450 nm with the Infinite M200Pro microplate reader (Tecan, Grödig, Austria). Data are shown as % inhibition of IgE binding to plate-bound apoAlt a 1.

Degranulation assay

Human FcεRI-expressing rat basophil cells (RBLsx38) (2×10^4/well in 96 well plates) were sensitized overnight with human serum of Alt a 1-allergic donors, 1:10 diluted in Tyrode's buffer. Sensitized cells were stimulated simultaneously with varying concentrations of *apo-* or *holo-*Alt a 1 in Tyrode's buffer for 1 h at 37 °C. Degranulation was assessed by measuring the released ß-hexosaminidase in the supernatant and of unreleased enzyme in the respective cell lysate. For this cell-free supernatant were collected and incubated with 200 μM 4-methylumbelliferyl-β-d- glucosaminide in 100 mM citric acid, pH 4.5 for 1 h at 37 °C, before terminating the enzymatic reaction by addition of 0.1 M glycine buffer, pH 10.7 and measuring cleaved fluorescence products at ex360/em452. As a positive control, cells were lysed with 0.2 % Triton X-100 to quantify the total β-hex content. The presented results were calculated as percentage release of total ß-hexosaminidase content[60].

Allergic sensitization model

Mice were randomized into 4 groups: apoAlt a 1 + DFO (n = 6), holoAlt a 1 + FeQ2 (n = 6), FeQ2 (n = 6), DFO (n = 6), and naïve (n = 2). Animals were pretreated 6 times on two consecutive days in 7 days intervals with 10 μg Alt a 1 + 0.5 nM DFO, 10 μg holoAlt a 1 + equimolar FeQ2, FeQ2 and 0.5 nM DFO in water respectively. One week after the last pretreatment mice were intraperitoneally (i.p.) sensitized twice with 5 μg Alt a 1 in 100 μl aluminium hydroxide (150 μl/mouse) in two weeks intervals. One week after the last i.p immunization, mice were i.p. challenged with 30 μg of Alt a 1. Body temperature as well as movements were monitored for 20 min after i.p. challenge using an Imaging system (Biomedical Int. R + D, Vienna)[74]. Rectal temperature was measured after 20 min. The allergic symptoms of challenged animals were scored as 0 points for no symptoms; 1 for scratching and rubbing around the nose and head, 2 for puffiness around the eyes and mouth, diarrhoea, pilar erection, reduced activity and/or decreased activity with increased respiratory rate; 3 for wheezing, laboured respiration, cyanosis around the mouth and the tail, and 4 for no activity after prodding or tremor and convulsion[37]. Blood and spleen cells were collected after euthanasia with CO_2 and sera were stored at − 80 °C until further processing.

In vitro stimulation of mice splenocytes

5×10^6 cells/ml isolated splenocytes of individual mice were treated with 10 μg/ml recombinant Alt a 1 or medium alone for 18 h at 37 °C/ 5% CO_2. Concanavalin A (2.5 μg/ml) was used as a positive control. Secreted mouse cytokines IL-10, IL-13 and IFNγ were measured with the corresponding commercial ELISAs (Invitrogen United States) based on the manufacturer's instructions.

Measurement of Alt a 1-specific antibodies in mouse serum by ELISA

Alt a 1-specific IgG1, IgG2a and IgE levels were measured by ELISA. Briefly, Alt a 1 4 μM/mL and serial dilutions of mouse IgG1, IgG2a, IgG2b and IgE standards were coated in carbonate buffer, blocked with 1 % BSA in PBS and incubated with diluted sera (1: 1000 for IgG1 and 1:100 IgG2a, IgG2b and 1:10 for IgE) overnight at 4 °C. Detection was performed by monoclonal rat anti-mouse IgG2a (clone R19-15), IgG2b (clone R12-3), IgA (clone c10-1), or IgE (clone R35-72) followed by polyclonal peroxidase-labelled goat anti-rat IgG (GE Healthcare). All primary antibodies were from BD Pharmingen. Tetramethylbenzidine (BD Bioscience) was used as substrate. The reaction was stopped with 1.8 M sulfuric acid and detected at 450 nm[75].

Flow cytometric analyses of mouse splenocytes

Single-cell suspensions of murine splenocytes (0.5×10^6 cells) were stained for monocytic cells using CD86-FITC (clone GL1), CD11c-PE (clone N418) and MHCII I-Ad-APC (cloneAMS-32.1) antibodies; for T-regulatory cells CD4-FITC (clone RM4-5), CD25-PE (clone PC61.5) and Foxp3-PE-Cy5 (clone FJK-16 s) antibodies were used according to the manufacturer's instructions. Antibodies were purchased from Bio-Legend or Thermo Fisher Scientific. Cells were acquired by flow cytometry (BD Bioscience Canto II). Gating on the living population and exclusion of doublets was done before gating for specific cell populations. Acquired cells were analysed using the FACSDiva Software 6.0[14].

Peripheral blood mononuclear cell stimulations

Peripheral blood mononuclear cells (PBMCs) from 26 Alternaria allergic subjects were isolated via Ficoll-Paque (GE Healthcare) and cells were washed with 0.9 % NaCl, before incubation with medium, 4 μM/mL apoAlt a 1, or holoAlt a 1-FeQ2, or or holoAlt a 1-Q2, in media neither with phenol red nor fetal calf serum for 18 h to ensure relative iron free-media conditions[46].

Flow cytometric analyses of human PBMCs

For analysis of the different cell populations, PBMCs were stained with a combination of the following antibodies in combination with Calcein Violet 450 AM (Thermo-Fisher). For T cells including Tregs: CD3-APC-Cy7 (Biolegend, clone SK7), CD4-PE-Cy7 (Biolegend, clone SK3), CD25-APC (Biolegend, clone BC96), CD127-PE (Biolegend, clone A019D5). For B-cells: CD19-APC-Cy7 (clone SJ25C1), CD27-PE (clone O323), CD38-APC (clone IT2.2).

Doublets were excluded, before gating on the living lymphocytic population. Acquisition and analysis were performed on a FACS Canto II machine (BD Bioscience, San Jose, CA, USA), recorded events were analysed with the FACSDiva Software 6.0.

Statistical analyses

Data were tested for normality before applying parametric or non-parametric tests. Matched data in Fig. 1 and Fig. 2DF were compared with RM one-way ANOVA followed by Holm-Sidak's multiple comparison test; for Fig. 2E RM-two-way ANOVA with Dunnett's multiple comparison test was employed. Mice clinical and antibody data were compared with ordinary one-way ANOVA and Tukey's multiple comparison test. Cytokine, and flow cytometric analysis were compared by ordinary one-way ANOVA following uncorrected Fisher's LSD with single pooled variance. Human PBMCs were compared by paired T test, except for Tregs, Th2 and plasma cells, in which Wilcoxon matched-pairs signed rank test was employed due to non-parametric distribution of the data.

All statistical tests were conducted with GraphPad Prism 10 Version for macOS (GraphPad, San Diego, CA, USA). All tests were two-sided and considered significant when p was less than 0.05.

CRediT authorship contribution statement

Aila Fakhimahmadi: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Karin Hufnagl:** Writing – review & editing, Supervision, Project administration, Investigation. **Ilir Hasanaj:** Writing – review & editing, Investigation. **Nathalie Szepannek:** Writing – review & editing. **Gerlinde Hofstetter:** Writing – review & editing, Project administration, Methodology, Investigation. **Markus Wiederstein:** Writing – review & editing, Visualization, Methodology, Investigation. **Sebastian A Jensen:** Writing – review & editing, Investigation. **Markus Berger:** Writing – review & editing, Investigation. **Markus Gorfer:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition. **Clara E. Pogner:** Writing – review & editing, Investigation, Funding acquisition. **Rodolfo Bianchini:** Writing – review & editing, Methodology. **Isabella Pali-Schöll:** Writing – review & editing, Investigation. **Erika Jensen-Jarolim:** Writing – review & editing, Funding acquisition. **Franziska Roth-Walter:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Funding

Danube Allergy Research Cluster DARC #08 (EJJ, FRW) and DARC #11(MG, CEP) of the Karl Landsteiner University, Krems, Austria.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jensen-Jarolim reports financial support was provided by Karl Landsteiner University, Krems, Austria. Franziska Roth-Walter reports financial support was provided by Karl Landsteiner University, Krems, Austria. Markus Gorfer reports financial support was provided by Karl Landsteiner University, Krems, Austria. Clara E Pogner reports financial support was provided by Karl Landsteiner University, Krems, Austria. Franziska Roth-Walter reports a relationship with Biomedical International R&D, Austria that includes: consulting or advisory. Roth-Walter reports a relationship with Allergy Therapeutics ATL, UK that includes: speaking and lecture fees. Roth-Walter reports a relationship with Bencard Allergie GmbH that includes: funding grants and speaking and lecture fees. Jensen-Jarolim reports a relationship with Biomedical International R&D that includes: equity or stocks. Roth-Walter has patent #EP2894478 issued to Biomedical Internation R&D. Jensen-Jarolim has patent #EP2894478 issued to Biomedical Internation R&D. no relationship or activity to declare If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mucimm.2025.11.006.

References

1. Lindblom SD, et al. Fungal Endophyte Alternaria tenuissima can Affect Growth and Selenium Accumulation in its Hyperaccumulator Host Astragalus bisulcatus. *Front Plant Sci*. 2018;9:1213.
2. DeMers M. Alternaria alternata as endophyte and pathogen. *Microbiology (Reading)*. 2022;168.
3. Kumari VV, et al. Plant Nutrition: an Effective Way to Alleviate Abiotic stress in Agricultural Crops. *Int J Mol Sci*. 2022;23.
4. Yu H. Studies on fungi of the normal skin. *Hifuka kiyo. Acta Dermatologica*. 1965;60:126–174.
5. Pastor FJ, Guarro J. Alternaria infections: laboratory diagnosis and relevant clinical features. *Clinical Microbiology and Infection*. 2008;14:734–746.
6. Vicki AM, Daniel JW. Alternaria: a Sinonasal Pathogen of Immunocompromised Hosts. *Clinical Infectious Diseases*. 1993;16:265–270.
7. Lo Porto D, et al. Phaeohyphomycosis in Solid Organ Transplant Recipients: a Case Series and Narrative Review of the Literature. *J Fungi (Basel)*. 2023;9.
8. Lee JY, Hyun M, Kim HA, Ryu SY. Unusual Presentation of Subcutaneous Phaeohyphomycosis by Alternaria alternate. *Ann Dermatol*. 2019;31:563–566.
9. Hattab Z, et al. Alternaria alternata infection causing rhinosinusitis and orbital involvement in an immunocompetent patient. *New Microbes New Infect*. 2019;32,100561.
10. Sanchez P, Velez-Del-Burgo A, Sunen E, Martinez J, Postigo I. Fungal Allergen and Mold Allergy Diagnosis: Role and Relevance of Alternaria alternata Alt a 1 Protein Family. *J Fungi (Basel)*. 2022;8.
11. Kespohl S, Raulf M. Mold Sensitization in Asthmatic and Non-asthmatic Subjects Diagnosed with Extract-based Versus Component-based Allergens. *Adv Exp Med Biol*. 2019;1153:79–89.
12. Hayes T, et al. Innate Immunity Induced by the Major Allergen Alt a 1 from the Fungus Alternaria is Dependent upon Toll-like Receptors 2/4 in Human Lung Epithelial Cells. *Front Immunol*. 2018;9:1507.
13. Roth-Walter F, et al. Nutrition in chronic inflammatory conditions: Bypassing the mucosal block for micronutrients. *Allergy*. 2024;79:353–383.
14. Blechert O, Xiong S, Chen J, Brand AC, Zhan P. Nutritional requirements of the human pathogenic fungus, Trichophyton rubrum, and nutritional immunity of the human skin as barrier against colonization. *Fungal Biology Reviews*. 2023;45,100330.
15. Fakhimahmadi A, et al. Mould allergen Alt a 1 spiked with the micronutrient retinoic acid reduces Th2 response and ameliorates Alternaria allergy in BALB/c mice. *Allergy*. 2024.
16. Sun J, Xiao S, Xue C. The tug-of-war on iron between plant and pathogen. *Phytopathol Res*. 2023;5:61.
17. Wu P-C, Chen Y-K, Yago JI, Chung K-R. Peroxisomes Implicated in the Biosynthesis of Siderophores and Biotin, Cell Wall Integrity, Autophagy, and Response to Hydrogen Peroxide in the Citrus Pathogenic Fungus Alternaria alternata. *Front Microbiol*. 2021;12.
18. Voß B, Kirschhöfer F, Brenner-Weiß G, Fischer R. Alternaria alternata uses two siderophore systems for iron acquisition. *Sci Rep*. 2020;10:3587.

19. Wu JJ, Wu PC, Yago JI, Chung KR. The Regulatory Hub of Siderophore Biosynthesis in the Phytopathogenic Fungus Alternaria alternata. *J Fungi (Basel)*. 2023;9.
20. Wu P-C, Choo Y-L, Wei S-Y, Yago JI, Chung K-R. Contribution of Autophagy to Cellular Iron Homeostasis and stress Adaptation in Alternaria alternata. *International Journal of Molecular Sciences*. 2024;25:1123.
21. Almeida RS, Wilson D, Hube B. Candida albicans iron acquisition within the host. *FEMS yeast research*. 2009;9:1000–1012.
22. Caza M, Kronstad JW. Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. *Front Cellular Infect Microbiol*. 2013;3:80.
23. Fakhimahmadi A, et al. Nutritional Provision of Iron Complexes by the Major Allergen Alt a 1 to Human Immune Cells Decreases its Presentation. *c*. 2023;24: 11934.
24. Fakhimahmadi A, et al. Nutritional Provision of Iron Complexes by the Major Allergen Alt a 1 to Human Immune Cells Decreases its Presentation. *Int J Mol Sci*. 2023;24.
25. Crawford A, Wilson D. Essential metals at the host–pathogen interface: nutritional immunity and micronutrient assimilation by human fungal pathogens. *FEMS Yeast Research*. 2015;15.
26. Roth-Walter F, et al. Nutrition in chronic inflammatory conditions: Bypassing the mucosal block for micronutrients. *Allergy*. 2023.
27. Roth-Walter F. Iron-Deficiency in Atopic Diseases: Innate Immune Priming by Allergens and Siderophores. *Front Allergy*. 2022;3, 859922.
28. Jin CW, et al. Iron deficiency-induced secretion of phenolics facilitates the reutilization of root apoplastic iron in red clover. *Plant Physiol*. 2007;144:278–285.
29. Luscher A, et al. Plant-Derived Catechols are Substrates of TonB-Dependent Transporters and Sensitize Pseudomonas aeruginosa to Siderophore-Drug Conjugates. *mBio*. 2022;13, e0149822.
30. Twaroch TE, et al. Predominant localization of the major Alternaria allergen Alt a 1 in the cell wall of airborne spores. *J Allergy Clin Immunol*. 2012;129:1148–1149.
31. Roth-Walter F, et al. Cow's milk protein beta-lactoglobulin confers resilience against allergy by targeting complexed iron into immune cells. *J Allergy Clin Immunol*. 2021; 147:321–334 e324.
32. Regner A, et al. Binding to Iron Quercetin Complexes increases the Antioxidant Capacity of the Major Birch Pollen Allergen Bet v 1 and Reduces its Allergenicity. *Antioxidants*. 2023;12:42.
33. Roth-Walter F, et al. The major cow milk allergen Bos d 5 manipulates T-helper cells depending on its load with siderophore-bound iron. *PLoS One*. 2014;9, e104803.
34. Roth-Walter F, et al. Bet v 1 from Birch Pollen is a Lipocalin-like Protein acting as Allergen only when devoid of Iron by promoting Th2 lymphocytes. *The Journal of biological chemistry*. 2014.
35. Kurup VP, Vijay HM, Kumar V, Castillo L, Elms N. IgE binding synthetic peptides of Alt a 1, a major allergen of Alternaria alternata. *Peptides*. 2003;24:179–185.
36. Twaroch TE, et al. Carrier-bound Alt a 1 peptides without allergenic activity for vaccination against Alternaria alternata allergy. *Clin Exp Allergy*. 2012;42:966–975.
37. Afify SM, et al. Bovine holo-beta-lactoglobulin cross-protects against pollen allergies in an innate manner in BALB/c mice: potential model for the farm effect. *Frontiers in Immunology*. 2021;12, 611474.
38. Lan F, Zhang N, Bachert C, Zhang L. Stability of regulatory T cells in T helper 2–biased allergic airway diseases. *Allergy*. 2020;75:1918–1926.
39. Avery DT, et al. Increased expression of CD27 on activated human memory B cells correlates with their commitment to the plasma cell lineage. *J Immunol*. 2005;174: 4034–4042.
40. Herzog CA, Muster HA, Li S, Collins AJ. Impact of congestive heart failure, chronic kidney disease, and anemia on survival in the Medicare population. *J Card Fail*. 2004;10:467–472.
41. Anand IS, Gupta P. Anemia and Iron Deficiency in Heart failure. *Circulation*. 2018; 138:80–98.
42. Philip KEJ, et al. The prevalence and associated mortality of non-anaemic iron deficiency in older adults: a 14 years observational cohort study. *Br J Haematol*. 2020;189:566–572.
43. Roth-Walter F, Pacios LF, Bianchini R, Jensen-Jarolim E. Linking iron-deficiency with allergy: role of molecular allergens and the microbiome. *Metallomics*. 2017;9: 1676–1692.
44. Vassilopoulou E, Venter C, Roth-Walter F. Malnutrition and Allergies: Tipping the Immune Balance towards Health. *J Clin Med*. 2024;13.
45. Petje LM, et al. Functional iron-deficiency in women with allergic rhinitis is associated with symptoms after nasal provocation and lack of iron-sequestering microbes. *Allergy*. 2021;76:2882–2886.
46. Bartosik T, et al. Ameliorating Atopy by Compensating Micronutritional Deficiencies in Immune Cells: a Double-Blind Placebo-Controlled pilot Study. *J Allergy Clin Immunol Pract*. 2022;10:1889–1902 e1889.
47. Haas H. Iron – a Key Nexus in the Virulence of Aspergillus fumigatus. *Frontiers in Microbiology*. 2012;3.
48. Yu Z, Xu C, Fang C, Zhang F. Causal effect of iron status on lung function: a Mendelian randomization study. *Front Nutr*. 2022;9, 1025212.
49. Brigham EP, McCormack MC, Takemoto CM, Matsui EC. Iron status is associated with asthma and lung function in US women. *PLoS One*. 2015;10, e0117545.
50. Abel-Fernandez E, Martinez MJ, Galan T, Pineda F. Going over Fungal Allergy: Alternaria alternata and its Allergens. *J Fungi (Basel)*. 2023;9.
51. Saenz-de-Santamaria M, Guisantes JA, Martinez J. Enzymatic activities of Alternaria alternata allergenic extracts and its major allergen (Alt a 1). *Mycoses*. 2006;49: 288–292.
52. Gong S, Tang J, Xiao Y, Li T, Zhang Q. The fungal effector AaAlta1 inhibits PATHOGENESIS-RELATED PROTEIN10-2-mediated callose deposition and defense responses in apple. *Plant Physiol*. 2024;197.
53. Salo PM, et al. Exposure to Alternaria alternata in US homes is associated with asthma symptoms. *J Allergy Clinical Immunol*. 2006;118:892–898.
54. Garrido-Arandia M, et al. Characterisation of a flavonoid ligand of the fungal protein Alt a 1. *Scientific Reports*. 2016;6:1–9.
55. Junier A, Weeks A, Alcaraz Y, Kumamoto Carol A. Bypass of Dfi1 Regulation of Candida albicans Invasive Filamentation by Iron Limitation. *mSphere*. 2022;7, e00779-00721.
56. Wang Z, et al. CYB561A3 is the key lysosomal iron reductase required for Burkitt B-cell growth and survival. *Blood*. 2021;138:2216–2230.
57. Ghatak SK, et al. Peanut protein sensitivity towards trace iron: a novel mode to ebb allergic response. *Food Chem*. 2015;176:308–313.
58. Jiang Y, et al. Recycled lithium battery nanomaterials as a sustainable nanofertilizer: Reduced peanut allergenicity and improved seed quality. *Sci Total Environ*. 2024; 955, 176900.
59. Tong P, et al. Iron-induced chelation alleviates the potential allergenicity of ovotransferrin in a BALB/c mouse model. *Nutr Res*. 2017;47:81–89.
60. Hufnagl K, et al. Retinoic acid-loading of the major birch pollen allergen Bet v 1 may improve specific allergen immunotherapy: in silico, in vitro and in vivo data in BALB/c mice. *Allergy*. 2020;75:2073.
61. Hufnagl K, et al. Retinoic acid prevents immunogenicity of milk lipocalin Bos d 5 through binding to its immunodominant T-cell epitope. *Sci Rep*. 2018;8:1598.
62. Low DW, Jamil A, Md Nor N, Kader Ibrahim SB, Poh BK. Food restriction, nutrition status, and growth in toddlers with atopic dermatitis. *Pediatr Dermatol*. 2020;37: 69–77.
63. Leung TF, et al. Assessment of dietary food and nutrient intake and bone density in children with eczema. *Hong Kong Med J*. 2017;23:470–479.
64. Kim SH, Lee JH, Ly SY. Children with atopic dermatitis in Daejeon, Korea: individualized nutrition intervention for disease severity and nutritional status. *Asia Pac J Clin Nutr*. 2016;25:716–728.
65. Xiang J, Wang H, Li T. Comorbidity of Vitamin A and Vitamin D Deficiency Exacerbates the Severity of Atopic Dermatitis in Children. *Dermatology*. 2019;235: 196–204.
66. Esenboga S, et al. Infantile atopic dermatitis: Serum vitamin D, zinc and TARC levels and their relationship with disease phenotype and severity. *Allergol Immunopathol (Madr)*. 2021;49:162–168.
67. Gomez HM, et al. Investigating the Links between lower Iron Status in Pregnancy and respiratory Disease in Offspring using Murine Models. *Nutrients*. 2021;13.
68. F. Roth-Walter *et al.*, in https://patents.google.com/patent/EP2894478B1/tr, E. P. office, Ed. (Austria, 2014), vol. EP2894478B1.
69. Chruszcz M, et al. Alternaria alternata allergen Alt a 1: a unique beta-barrel protein dimer found exclusively in fungi. *J Allergy Clin Immunol*. 2012;130:241–247 e249.
70. Word JM, Lovell SC, Richardson JS, Richardson DC. Asparagine and glutamine: using hydrogen atom contacts in the choice of side-chain amide orientation. *J Mol Biol*. 1999;285:1735–1747.
71. Ravindranath PA, Forli S, Goodsell DS, Olson AJ, Sanner MF. AutoDockFR: advances in Protein-Ligand Docking with Explicitly Specified Binding Site Flexibility. *PLoS Comput Biol*. 2015;11, e1004586.
72. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J Chem Inf Model*. 2021;61:3891–3898.
73. Pettersen EF, et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J Computat Chem*. 2004;25:1605–1612.
74. Roth-Walter F, et al. Pasteurization of milk proteins promotes allergic sensitization by enhancing uptake through Peyer's patches. *Allergy*. 2008;63:882–890.
75. Afify SM, et al. Micronutritional supplementation with a holoBLG-based FSMP (food for special medical purposes)-lozenge alleviates allergic symptoms in BALB/c mice: Imitating the protective farm effect. *Clinical & Experimental Allergy*. 2022;52: 426–441.